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Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle

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ABSTRACT: The interaction between sarcomere length and postmortem proteolysis as related to meat tenderness is not clear. The extent of thick and thin filament overlap alters actomyosin binding and may alter substrate availability during aging-induced tenderization. The objective of this study was to determine the influence of sarcomere length on proteolytic degradation in beef. Strips from bovine semitendinosus were either stretched 40% and restrained or allowed to shorten unrestrained in an ice bath. After rigor completion, 0.6-cm cross sections were fabricated and were randomly assigned to 2, 4, 7, or 10 d of aging treatments. Myofibrils were isolated for sarcomere length determination. Samples were collected and frozen for shear force analysis, and muscle proteins were extracted for SDS-PAGE and Western blotting analyses to determine troponin T (TnT) proteolysis. Sarcomere length was greater ($P < 0.01$) in stretched muscle samples compared with shortened samples (2.57 vs. 1.43 μm , respectively). Correspondingly, shear force values were

greater ($P < 0.05$) in shortened samples than stretched samples. Western blots revealed the presence of 3 major intact TnT bands that diminished with time postmortem and 4 bands (TnT degradation products) that accumulated during postmortem storage. Quantification of intact TnT showed increased ($P < 0.05$) proteolysis at 4 and 7 d postmortem in samples with long sarcomeres. By 10 d, only traces of the greatest molecular weight intact TnT band were evident in both shortened and stretched samples, suggesting this TnT band may be more susceptible to proteolysis than other intact TnT bands. Degradation products of TnT appeared earlier postmortem in samples with long sarcomeres. The 30-kDa TnT fragment appeared after 7 d of postmortem storage in samples with long sarcomeres but not until 10 d in muscle containing short sarcomeres. Collectively, these data show that postmortem TnT proteolysis is sarcomere length-dependent and suggest that thick and thin filament overlap may influence the postmortem aging process in beef.

Key words: tenderness, sarcomere length, proteolysis, troponin-T, beef

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INTRODUCTION

Variation in meat tenderness remains one of the most critical quality problems facing the beef industry. Many studies have demonstrated that tenderness, or lack thereof, greatly affects the product satisfaction and purchasing decisions of consumers when buying fresh beef (Dransfield, 1985; Miller et al., 1995; Boleman et al., 1997; Lusk et al., 2001; Shackelford et al., 2001). To provide more consistently tender beef and improve the current grading system, a clearer understanding of the mechanisms regulating tenderization is required.

The independent contributions of sarcomere length and postmortem proteolysis to tenderness are well-established. Classic work by Marsh and Carse (1974) and Herring et al. (1965) demonstrated that muscles with longer sarcomere lengths have lower resistance to shear force. Additionally, many studies have shown that degradation of muscle proteins by the calpain system during refrigerated storage improves meat tenderness (Goll et al., 1992; Koohmaraie, 1996; Koohmaraie and Geesink, 2006). Thus, the ultimate shear force value of meat reflects the balance between sarcomere length and the extent of postmortem proteolysis (Jiang, 1998). Unfortunately, the interaction between these 2 elements is unclear. Wheeler and Koohmaraie (1994) proposed that increased overlap of the thick and thin filaments in short sarcomeres may reduce the availability of proteolytically susceptible sites. Conversely, several studies have concluded that sarcomere length does not affect the extent of proteolysis (Koohmaraie et

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al., 1984; Jaime et al., 1992; Wheeler and Koohmaraie, 1999). However, these studies compared cold-shortened and normal longissimus muscle. Wheeler and Koohmaraie (1999) reported a range in sarcomere lengths from 1.36 μm (cold-shortened longissimus) to 1.69 μm (normal longissimus). If the degree of filament overlap alters the availability of substrates for proteolysis, this limited range in sarcomere length, especially at extremely short lengths, likely fails to provide a range sufficient to elicit differences in proteolysis. Given that thick filament length is 1.5 to 1.6 μm , (Page and Huxley, 1963; Bendall and Voyle, 1967) and thin filaments measure 1.3 μm in each half sarcomere (Ringkob et al., 2004), the number of actomyosin bonds differs little between cold-shortened muscle and stretched muscle unless sarcomeres in stretched muscle measure 2.6 to 2.7 μm . Thus, data from studies with a limited range in sarcomere lengths may be misleading. Therefore, the objective of this study was to investigate the effect of sarcomere length on postmortem proteolysis in beef semitendinosus with a wide range of sarcomere lengths, which allow for differences in the extent of overlap between the thick and thin filaments.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because the samples were obtained from a state-inspected slaughter facility (Purdue University Meat Laboratory).

Muscle Samples

Two A-maturity market heifers were slaughtered at the Purdue University Meat Science Teaching and Research Laboratory using standard slaughtering procedures. Approximately 20 min postmortem, semitendinosus muscles were removed from each carcass. External fat was removed, and strips measuring approximately 5 cm in diameter and 20 cm in length were dissected along the long axis of the muscle, parallel to muscle fiber orientation. All strips were removed from the superficial portion of the muscle. To generate long sarcomeres, strips were clamped to adjustable supports and stretched to a length of 40% greater than the excised muscle length. Each strip was wrapped in plastic wrap to prevent dehydration and was held at 4°C for 24 h. To generate short sarcomeres, unrestrained strips were sealed in a plastic bag and placed in an ice bath for 24 h. Upon rigor completion, an approximately 7.5-cm section was removed from the center of each strip. From this section, two 2.54-cm-thick steaks were removed and four 0.6-cm slices were randomly made from each strip. To determine if sarcomere length was different along the length of each 7.5-cm section, sarcomere length was determined on one half of each 0.6-cm thick slice at 2 d. The remaining half of each slice was vacuum-packaged and aged until 2, 4, 7, or 10 d postmortem and then was frozen at -40°C for SDS-PAGE and Western blotting analysis of whole

muscle extracts. Pairs of steaks (2.54 cm thick) were vacuum-packaged, aged until either 2 or 10 d postmortem at 4°C, and frozen at -40°C for Warner-Bratzler shear force analysis.

Myofibril Preparation

At 2 d postmortem, myofibrils were purified according to a modification of the procedure of Swartz et al. (1993). Approximately 5 g of muscle was minced with a scalpel and homogenized in 35 mL of rigor buffer (RB, 75 mM KCl, 10 mM imidazole, 2 mM MgCl_2 , 2 mM ethylene glycol tetraacetic acid, 1 mM NaN_3 , pH 7.2) for two 15-s bursts using a Polytron homogenizer (Brinkman Instruments, New York, NY) at medium speed (speed setting 6). The suspension was centrifuged at $1,000 \times g$ for 10 min at 4°C. The supernatant was decanted, and the remaining pellet was homogenized in 35 mL of RB for two 15-s bursts at medium speed. The suspension was centrifuged again at $1,000 \times g$ for 10 min. The pellet was resuspended in 35 mL of RB and centrifuged at $1,000 \times g$ for 10 min. Final myofibril pellets were resuspended in 20 mL of RB plus 0.1 mM phenylmethylsulfonyl fluoride and diluted by addition of an equal volume of glycerol. Isolated myofibrils were stored at -20°C.

Sarcomere Length Determination

Twenty microliters of the myofibril preparation was spread on a coverslip (No. 1 1/2, Corning Inc., Lowell, MA) and fixed with 3% (vol/vol) formaldehyde in RB. Samples were incubated at 35°C for 10 min. Fixative was decanted, and samples were mounted with 30 μL of mounting media [75 mM KCl, 10 mM Tris (pH 8.5), 2 mM MgCl_2 , 2 mM ethylene glycol tetraacetic acid, 1 mM NaN_3 , 1 mg/ml *p*-phenylenediamine, and 75% (vol/vol) glycerol]. Sarcomere length was measured by adapting the procedure of Warner et al. (1997). Sarcomeres were measured directly using phase contrast microscopy (Nikon Microphot FXL, Nikon Corp., Melville, NY) and a 100 \times oil immersion objective. Images were captured using a Sony color video camera (model DXC-151A, Sony Corp., Tokyo, Japan). The first 20 myofibrils observed that were positioned in a straight-line orientation and had at least 5 full sarcomeres in length were evaluated for sarcomere length. A line was drawn across 5 sarcomeres using a measuring tool (Scion imaging software, Scion Corp., Frederick, MD), and the average sarcomere length was recorded.

Warner-Bratzler Shear Force Determination

Frozen 2.54-cm-thick steaks were equilibrated to 4°C before cooking. Steaks were placed in a plastic bag, immersed in a circulating water bath (85°C), and cooked to an internal temperature of 71°C. At least 9 cores (1.27-cm diam.) were removed from each pair of steaks and were sheared perpendicular to fiber orientation using a meat shear blade (1.8-mm thick) mounted to

a texture analyzer (Model TMS-90, Food Technology Corp., Sterling, VA). Peak shear force values (kg) were recorded.

Whole Muscle Protein Extraction

For samples from 2, 4, 7, and 10 d of postmortem aging, proteins were extracted as described by Huff-Loneragan et al. (1996b), with slight modifications. Briefly, 0.2 g of muscle was minced and added to 25 volumes of homogenizing solution [10 mM sodium phosphate and 10% (wt/vol) SDS, pH 7.0]. Samples were homogenized using a motor-driven Dounce homogenizer and clarified by centrifugation at $1,500 \times g$ for 15 min at 4°C. Protein concentration was determined using the RC/DC Protein assay (based on the Lowry assay; Bio-Rad Laboratories, Hercules, CA), and samples were diluted with water to a final concentration of 6.4 mg/mL. One volume of each sample was added to 0.5 volumes of sample buffer [3 mM EDTA, 3% (wt/vol) SDS, 30% (vol/vol) glycerol, 0.003% (wt/vol) pycronin Y, and 30 mM Tris-HCl pH 8.0] and 0.1 volumes of 2-mercaptoethanol, for a final protein concentration of 4.0 mg/mL. Samples were immediately heated to 100°C for 5 min and stored at -20°C for 2 to 8 wk.

Gel Electrophoresis and Transfer Conditions

Denatured protein samples were thawed, and 1 volume of each was further diluted with 4 volumes of sample buffer and 0.1 volumes of 2-mercaptoethanol. Five microliters of each diluted muscle extract (3.9 µg of total protein/lane) was loaded on 15% polyacrylamide resolving gels (Ready Gel, Tris-HCl gels, Bio-Rad Laboratories). In addition, a broad range (200 to 6.5 kDa) of biotinylated molecular weight (MW) standard (1.2 µg/lane; Bio-Rad Laboratories) was loaded onto each gel. Gels were run on the Bio-Rad Criterion Cell system at constant 200 V for 80 min at 4°C. The running buffer used in both the upper and lower chamber consisted of 25 mM Tris, 192 mM glycine, 2 mM EDTA, and 0.1% (wt/vol) SDS. After electrophoresis, gels were equilibrated for 30 min at room temperature in transfer buffer [25 mM Tris, 192 mM glycine, and 15% (vol/vol) methanol]. Gels were transferred to polyvinylidene difluoride membranes at 4°C using a Criterion blotter (Bio-Rad Laboratories) at a constant 90 V for 45 min.

Western Blotting

After transfer, membranes were blocked overnight at 4°C in blocking solution [PBS + 1% (vol/vol) Tween 20 (PBST) and 5% nonfat dry milk]. Blots were washed 3 times (10 min per wash) in PBST and then were incubated for 90 min at room temperature with the primary antibody, which was monoclonal anti-troponin T (TnT; JLT-12, Sigma, St. Louis, MO) diluted 1:30,000 in PBST containing 1% (wt/vol) bovine serum albumin. Blots were washed 3 times with PBST (10 min per

wash) and were then incubated with a goat anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (Bio-Rad Laboratories) diluted 1:25,000 (vol/vol) in PBST for 60 min at room temperature. Blots were washed 3 times in PBST, and bands were visualized using an Amplified Opti-4CN substrate kit (Bio-Rad Laboratories). Immunoreactive TnT bands were identified, and the disappearance of intact TnT was quantified using Kodak 1D software (Eastman Kodak, Rochester, NY). To account for potential variation in protein loading, intensities of protein bands were expressed relative to the total intensity of all the protein bands within the lane. The relative intensity of bands was then normalized to the relative intensity of intact TnT at 2 d. Five blots were analyzed per treatment by animal combination.

Statistical Analysis

Shear force and blot data were evaluated using a mixed model analysis (PROC MIXED, SAS Inst. Inc., Cary, NC) with repeated measures. The model included treatment (stretched vs. shortened muscle) and aging (2, 4, 7, or 10 d) as fixed effects and animal as a random block effect. Treatment means were separated using a least squares difference test with the significance level set at $P < 0.05$.

RESULTS

Sarcomere Length and Warner-Bratzler Shear Force

Sarcomere length was greater ($P < 0.01$) in stretched versus shortened muscle strips (2.57 vs. 1.43 µm), respectively. No differences in sarcomere length were observed along the length of each muscle strip (Table 1). Shear force values (Table 2) were lower ($P < 0.05$) for stretched samples compared with shortened samples at both 2 and 10 d of aging. Aging reduced ($P < 0.0001$) shear forces values in shortened samples; however, no significant improvement was achieved with aging in stretched samples.

Western Blotting

Figure 1 shows a representative Western blot of protein extracts labeled with a monoclonal antibody to TnT. The biotinylated MW standard was used to estimate the MW of each immunoreactive TnT band. Bands 1 to 3 (42, 41, and 40 kDa, respectively) correspond to intact TnT, because these bands decrease in intensity over time. Bands 4 to 7 (38, 37, 33, and 30 kDa, respectively) appear and increase in intensity with increasing time postmortem and therefore correspond to proteolytic degradation fragments of TnT. Visual assessment of blots showing both treatments over the entire aging period demonstrate that intact TnT (bands 1 to 3) decreased in abundance with time postmortem in samples with short and long sarcomeres, whereas the

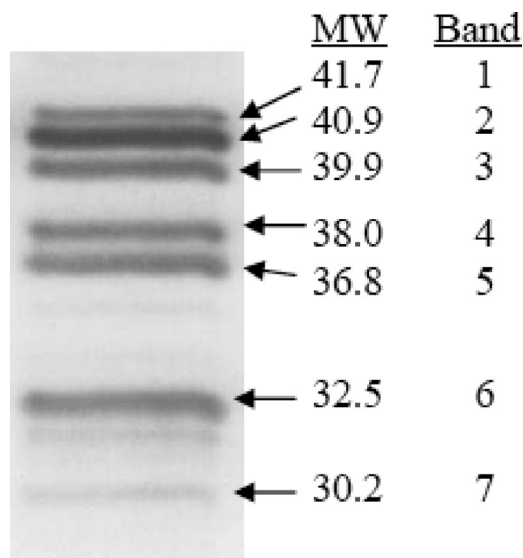


Figure 1. Molecular weights (MW, kDa) of troponin T immunoreactive bands.

relative abundance of the 4 proteolytic TnT fragments (bands 4 to 7) increased over time (Figure 2).

Differences in the degradation pattern of intact TnT were observed between samples with long and short sarcomeres. Figure 3 quantifies the disappearance of intact TnT (sum abundance of bands 1 to 3) in samples containing long and short sarcomeres throughout the 10-d aging period. Samples with shorter sarcomeres had a greater amount ($P < 0.05$) of intact TnT through 7 d of aging. By 10 d postmortem, however, the amount of intact TnT present did not differ with sarcomere length. Interestingly, the greatest MW intact TnT band (42 kDa) appeared to degrade more rapidly than the 2 smaller intact bands (41 and 40 kDa, respectively) as shown in Figure 2. Only traces of band 1 (42 kDa) were evident by 10 d postmortem in either treatment.

Corresponding to the disappearance of intact TnT, the lower MW TnT fragments appeared earlier in samples with longer sarcomeres (Figure 2). A qualitative analysis of these degradation fragments reveals bands 4 and 5 (38 and 37 kDa, respectively) appeared by 4 d in samples with long sarcomeres but were not evident in shortened samples until 10 d postmortem. In stretched samples, band 6 appeared by 4 d and remained pronounced at 10 d. In samples with short sarcomeres, however, band 6 was barely detectable at 4 d. Band 7 (30 kDa) appeared by 7 d in samples with long sarcomeres but was not detected by the TnT antibody until 10 d postmortem in shortened samples.

DISCUSSION

Sarcomere length (Herring et al., 1965; Hostetler et al., 1972; Davis et al., 1979) and postmortem proteolysis (Goll et al., 1995; Koohmaraie, 1996) have long been recognized as factors influencing meat tenderness; however, the interaction between these critical

Table 1. Least squares means for sarcomere length of stretched and shortened beef semitendinosus muscle samples at 2 d postmortem and designated for 2, 4, 7, or 10 d of aging (pooled SEM = 0.05)

Treatment	Aging period, ¹ d	Sarcomere length, ² μ m
Stretched	2	2.58 ^a
	4	2.55 ^a
	7	2.56 ^a
	10	2.59 ^a
Shortened	2	1.50 ^b
	4	1.40 ^b
	7	1.42 ^b
	10	1.39 ^b
P-value for treatment		0.0032

^{a,b}Means without a common superscript differ ($P < 0.0001$).

¹Sarcomere length of samples designated for 2, 4, 7, and 10 d of aging was measured at 2 d postmortem to determine variation along the length of the muscle strip.

²n = 20 myofibrils containing >5 intact sarcomeres.

determinants of tenderness remains controversial. The present study focused on defining the interaction between sarcomere length and the extent of postmortem proteolysis in beef semitendinosus using a model that generated a wider range of sarcomere lengths than those used in previous studies. The protocol generated sarcomere lengths of 2.57 and 1.43 μ m for stretched and shortened muscle strips, respectively. This wide range of sarcomere lengths allows us to study more effectively the influence of sarcomere length on proteolysis. Some investigators (Henrickson and Mjoseth, 1964; Reuter et al., 2002) have demonstrated that intramuscular differences in shear force values exist within the semitendinosus. Because this change may be due to unaccounted for sarcomere length differences, we documented that sarcomere length did not differ across the length of the semitendinosus strips used in this study (Table 1).

It is possible that the approach used to shorten muscle altered proteolytic enzyme activity. Some have shown that low temperatures during rigor onset reduces calpain-mediated proteolysis in beef muscle, thereby limiting tenderization during the postmortem storage (Dransfield, 1994; King et al., 2003). Such data argue that muscles in the current study were not capable of normal proteolysis, regardless of sarcomere length. However, Koohmaraie (1992) suggested that low temperatures during rigor development decrease the rate

Table 2. Least squares means (\pm SE) for Warner-Bratzler shear force of stretched and shortened beef semitendinosus muscle samples after 2 or 10 d of aging

Treatment	Aging period, d	Shear force, kg
Stretched	2	5.49 ^c \pm 1.26
	10	4.59 ^c \pm 1.25
Shortened	2	13.58 ^a \pm 1.27
	10	10.91 ^b \pm 1.27

^{a-c}Means without a common superscript differ ($P < 0.001$).

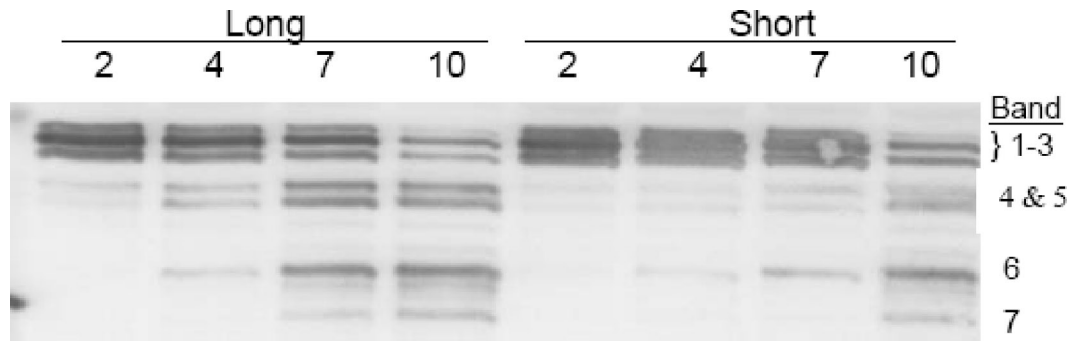


Figure 2. Representative Western blot of whole muscle protein extracts from bovine semitendinosus muscle with long (mean sarcomere length = 2.57 μm) and short (mean sarcomere length = 1.43 μm) sarcomeres. Postmortem aging periods for each sample were 2, 4, 7, or 10 d. Blots were labeled with monoclonal anti-troponin T (clone JLT-12, Sigma, St. Louis, MO). Bands are as indicated in Figure 1.

of autolytic inactivation of μ -calpain allowing extended activity postmortem. In the current experiment, samples with long sarcomeres reached 10°C by 165 min postmortem and 3°C by 225 min postmortem. Similarly, samples with short sarcomeres reached 10°C by 115 min postmortem and 0°C by 150 min postmortem (data not shown). Thus, given that samples from both treatments likely experienced cold-shortening conditions, differences in proteolysis in our study were more likely due to the influence of ultrastructural changes between long and short sarcomeres and not due to variations in endogenous protease activity. We, however, cannot rule out the latter.

The finding that samples containing long sarcomeres had lower shear force values than samples with short sarcomeres is consistent with others reporting a posi-

tive correlation between improved tenderness and sarcomere length (Herring et al., 1967; Marsh et al., 1974). Aging decreased shear force in samples with shorter sarcomeres from 13.58 kg at 2 d postmortem to 10.91 kg at 10 d of postmortem storage (~20% improvement), indicating that shortened beef muscle retains the capacity to tenderize with storage. By 10 d postmortem, however, these samples failed to reach the equivalent shear force values of stretched samples. This suggests that although aging-induced improvements in tenderness can occur in shortened muscle samples, thick and thin filament overlap remains one of the most critical determinants of meat tenderness. In contrast, samples with longer sarcomeres had reduced shear force values at 2 d postmortem and did not experience a significant reduction in shear force with 10 d of aging. This obser-

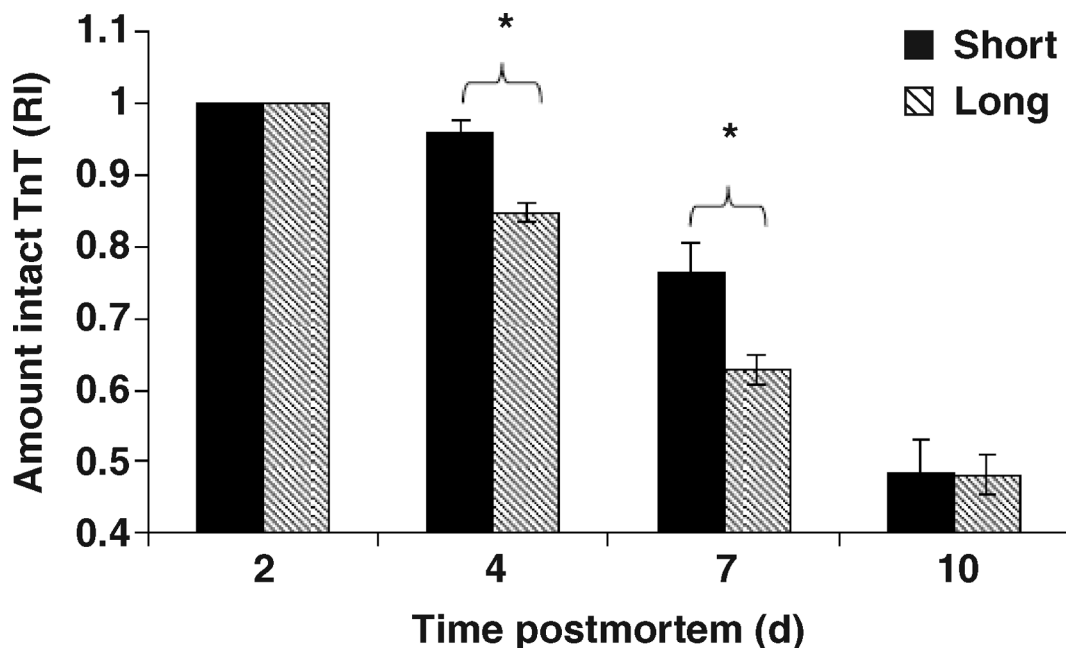


Figure 3. Disappearance of intact troponin T (TnT; bands 1 to 3) in whole muscle protein extracts from bovine semitendinosus muscle with long and short sarcomeres. Abundance of intact TnT for all aging periods is expressed relative to the abundance of intact TnT at 2 d postmortem. Bars represent mean \pm SE; * P < 0.05.

vation is consistent with Huff-Lonergan et al. (1996a), who also observed no significant decrease in shear force values of tender samples until 28 d of storage. Therefore, if samples were aged for an extended period of time, additional improvements may have occurred. Although samples with long sarcomeres exhibited little degradation of TnT after only 2 d of aging, it is possible that early postmortem (<2 d) changes in structural proteins such as titin and nebulin could have contributed to the low shear force of these samples. It is also possible that stretched muscle samples reached a threshold of background toughness (given the high amount of connective tissue in this muscle), and although proteolysis is occurring as reflected by Western blotting, it is not translated to changes in shear force. Thus, in this study, the extreme differences in sarcomere length and the number of actin-myosin crossbridges seem to have a more substantial effect on tenderness than proteolysis.

Western blotting analyses revealed 7 immunoreactive TnT bands. Bands 1 to 3 most likely represent isoforms of the intact protein with MW of approximately 42, 41, and 40 kDa. These results are in line with other investigators reporting the MW of intact TnT as 40 kDa (Negishi et al., 1996; Hughes et al. 2001). Muroya et al. (2006) reported the presence of 4 bovine fast TnT isoforms in beef longissimus with MW of 36.5, 35.4, 34.8, and 32.8 kDa. In the current study, we were not able to discern the fourth intact TnT band but understand that differences may be due to differences in the muscles or blotting protocols, or both, used in each study. Additionally, the estimated MW of bands 1 to 3 are approximately 5 kDa greater than those identified by Muroya et al. (2006). This difference is likely due to variation in gel systems and MW standards. Only traces of the greatest MW TnT isoform were detectable by 10 d postmortem in both long and short samples. The lower MW intact TnT bands also experienced proteolysis, but substantially more immunoreactive TnT was detected in these bands at 10 d postmortem than band 1. These data suggest that TnT isoforms may be differentially susceptible to postmortem proteolysis and argue that muscle-to-muscle differences observed during the aging process may be partially due to differences in the relative amount and types of isoforms present for various tenderness-related proteins. For example, isoforms of cytoskeletal proteins such as titin and nebulin may also be differentially susceptible to degradation by proteases. Due to their structural function, changes in the rate or extent of proteolysis of these proteins could result in tenderness variation.

Consistent with others (Negishi et al., 1996; Muroya et al., 2004), we also identified the presence of TnT degradation products ranging from 38 to 30 kDa. The increase in the abundance of these immunoreactive fragments corresponds with the decrease in the amount of intact TnT. Furthermore, degradation products appeared earlier in samples with longer sarcomeres supporting our hypothesis that sarcomere length influ-

ences postmortem proteolysis in beef. The appearance of bands 4 to 7 was not quantified, because this is not an adequate representation of the degradation of TnT. Due to the nature of proteolytic degradation, some fragments may not be detected using immunoblotting and therefore would not be represented in quantitative analysis.

Although TnT is classified as a regulatory protein and likely does not directly influence tenderness, degradation during postmortem storage to a 30-kDa fragment is well-documented (Ho et al., 1994; Negishi et al., 1996). Appearance of this fragment increases with postmortem tenderization and is considered a good marker for proteolysis (Penny and Dransfield, 1979). Additionally, some have suggested that degradation of TnT may indirectly improve tenderness through disruption of thin filament integrity and thick and thin filament interactions (Ho et al., 1994; Huff-Lonergan et al., 1996a). The role sarcomere length plays in regulating the degradation of this protein is largely unknown but intriguing. Our data show intact TnT degradation occurs earlier postmortem in samples with longer sarcomeres. Data also indicate proteolysis occurs in samples with short sarcomeres, albeit to a lesser extent, at least initially. The filamentous structure of muscle inherently gives rise to a tough product; therefore, any disruption of this architecture should result in more tender meat. Of the proteases endogenous to skeletal muscle, the calpain system (specifically μ -calpain) is the most likely candidate to create tenderness-related changes during postmortem storage (Koochmaraie, 1996); however, the influence of other protease systems has been recently proposed (Kemp et al., 2006). Calpains are intracellular proteases that associate with the myofibrillar structure, primarily in close proximity to the Z-disc. Only small amounts of calpains are found localized in the I-band and traces in the A-band (Kumamoto et al., 1992; Goll et al., 2003). Given that calpains localize at some distance from many potential substrates throughout the entire span of the sarcomere (Koochmaraie, 1996), it is possible that during postmortem storage, these enzymes must diffuse throughout the sarcomeric structure to access substrates. In the case of short sarcomeres, the thick filament and associated rigor bonds would be closer to the Z-line, potentially in direct contact. This dense matrix could inhibit the migration of calpains to their substrates thus retarding postmortem proteolysis but not inhibiting the process. Similarly, the portion of the thin filament located close to the Z-line in longer sarcomeres would not be associated with the thick filament resulting in minimal obstruction and easier access to substrates in this region, such as TnT. Along these same lines yet mechanistically different is the possibility that rigor bonds alter access to proteolytic cleavage sites on TnT. Shorter sarcomeres have a greater proportion of actomyosin bonds; therefore, the physical interaction of this structure with TnT may limit proteolysis early postmortem. Future studies comparing the degrada-

tion patterns of purified troponin and TnT within the myofibrillar structure could help determine the validity of this hypothesis.

Another alternative explanation for delayed TnT proteolysis in samples with short sarcomeres is that the proteolytic cleavage sites are obscured by other proteins within the tightly packed ultrastructure. Large cytoskeletal proteins such as titin and nebulin may limit access or perhaps partially block substrate cleavage sites due to folding or crimping in the shortened sarcomere. For example, titin is associated with the thick filament and is ~1 μm in length, spanning the half-sarcomere, from Z-line to M-line (Trinick, 1991). If the entire sarcomere length is <2.0 μm , this protein would have to contort in some manner to remain in the sarcomere. Such changes could hinder the ability of proteases to access their substrates. Regardless, data from the current study argue that proteolysis is at least partially regulated by sarcomere length. Additional studies are necessary to determine the specific mechanism responsible for differences in proteolysis of samples with long and short sarcomeres. Future work into the effect of sarcomere length on proteolysis of cytoskeletal proteins could also provide insight into this interaction.

In conclusion, postmortem degradation of intact TnT occurred earlier postmortem in bovine semitendinosus samples with long sarcomeres, suggesting protein interactions within the sarcomeric structure influence postmortem proteolysis. Appearance of TnT degradation products also occurred earlier in samples with long sarcomeres. The regulation of postmortem beef tenderization remains an unfolding story; however, these data provide evidence that the interaction between sarcomere length and proteolysis influences this phenomenon. Thus, future studies should account for the influence of sarcomere length on the rate and extent of proteolysis.

LITERATURE CITED

- Bendall, J. R., and C. A. Voyle. 1967. A study of the histological changes in the growing muscles of beef animals. *J. Food Technol.* 2:259–283.
- Boleman, S. J., S. L. Boleman, R. K. Miller, J. F. Taylor, H. R. Cross, T. L. Wheeler, M. Koohmaraie, S. D. Shackelford, M. F. Miller, R. L. West, D. D. Johnson, and J. W. Savell. 1997. Consumer evaluation of beef of known categories of tenderness. *J. Anim. Sci.* 75:1521–1524.
- Davis, G. W., G. C. Smith, Z. L. Carpenter, T. R. Dutson, and H. R. Cross. 1979. Tenderness variations among beef steaks from carcasses of the same USDA quality grades. *J. Anim. Sci.* 49:103–114.
- Dransfield, E. 1985. Evidence of consumer reaction to meat of different origins. Page 45 in *The Long-Term Definition of Meat Quality: Controlling the Variability of Quality in Beef, Veal, Pigeat and Lamb*. G. Harrington, ed. European Communities, Brussels, Belgium.
- Dransfield, E. 1994. Modelling post-mortem tenderisation—V: Inactivation of calpains. *Meat Sci.* 37:391–409.
- Goll, D. E., R. G. Taylor, J. A. Christiansen, and V. F. Thompson. 1992. Role of proteinases and protein turnover in muscle growth and meat quality. Pages 25–36 in *Proc. 44th Annual Reciprocal Meat Conference*, Manhattan, KS. Natl. Livest. Meat Board, Chicago, IL.
- Goll, D. E., R. G. Taylor, and V. F. Thompson. 1995. Does proteolysis cause all the postmortem tenderization or are changes in actin/myosin interaction involved? Pages 537–544 in *Proc. 41st International Congress of Meat Science and Technology*, San Antonio, TX. Meat Sci. Assoc., Natl. Livest. Meat Board, Chicago, IL.
- Goll, D. E., V. F. Thompson, H. Li, W. Wei, and J. Cong. 2003. The calpain system. *Physiol. Rev.* 83:731–801.
- Henrickson, R. L., and J. H. Mjoseth. 1964. Tenderness variation in two bovine muscles. *J. Anim. Sci.* 23:325–328.
- Herring, H. K., R. G. Cassens, and E. J. Briskey. 1965. Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. *J. Food Sci.* 30:1049–1054.
- Herring, H. K., R. G. Cassens, G. G. Suess, V. H. Brungardt, and E. J. Briskey. 1967. Tenderness and associated characteristics of stretched and contracted bovine muscles. *J. Food Sci.* 32:317–323.
- Ho, C. Y., M. H. Stromer, and R. M. Robson. 1994. Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin-T. *Biochimie* 76:369–375.
- Hostetler, R. L., B. A. Link, W. A. Landmann, and H. A. Fitzhugh. 1972. Effect of carcass suspension of sarcomere length and shear force of some major bovine muscles. *J. Food Sci.* 37:132–135.
- Huff-Lonerger, E., T. Mitsuhashi, D. D. Beekman, F. C. Parrish Jr., D. G. Olsen, and R. M. Robson. 1996a. Proteolysis of specific muscle structural proteins by micro-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *J. Anim. Sci.* 74:993–1008.
- Huff-Lonerger, E., T. Mitsuhashi, F. C. Parrish Jr., and R. M. Robson. 1996b. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting comparisons of purified myofibrils and whole muscle preparations for evaluating titin and nebulin in postmortem bovine muscle. *J. Anim. Sci.* 74:779–785.
- Hughes, M. C., S. Geary, E. Dransfield, P. L. H. McSweeney, and E. E. O'Neill. 2001. Characterization of peptides released from rabbit skeletal muscle troponin-T by μ -calpain under conditions of low temperature and high ionic strength. *Meat Sci.* 59:61–69.
- Jaime, I., J. A. Beltran, P. Cena, P. Lopez-Lorenzo, and P. Roncales. 1992. Tenderisation of lamb meat: Effect of rapid postmortem temperature drop on muscle conditioning and aging. *Meat Sci.* 32:357–366.
- Jiang, S. T. 1998. Contribution of muscle proteinases to meat tenderization. *Proceedings of the National Science Council, ROC Part B. Life Sci.* 22:97–107.
- Kemp, C. M., R. G. Bardsley, and T. Parr. 2006. Changes in caspase activity during the postmortem conditioning period and its relationship to shear force in porcine longissimus muscle. *J. Anim. Sci.* 84:2841–2846.
- King, D. A., M. E. Dikeman, T. L. Wheeler, C. L. Kastner, and M. Koohmaraie. 2003. Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. *J. Anim. Sci.* 81:1473–1481.
- Koohmaraie, M. 1992. Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle μ -calpain. *J. Anim. Sci.* 70:3071–3080.
- Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Sci.* 43:193–201.
- Koohmaraie, M., and G. H. Geesink. 2006. Contribution of post-mortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Sci.* 74:34–43.
- Koohmaraie, M., W. H. Kennick, A. F. Anglemier, E. A. Elgasim, and T. K. Jones. 1984. Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDS-polyacrylamide gel electrophoresis. *J. Food Sci.* 49:290–291.
- Kumamoto, T., W. C. Kleese, J. Y. Kong, D. E. Goll, P. R. Pierce, and R. E. Allen. 1992. Localization of the Ca^{2+} -dependent pro-

- teinases and their inhibitor in normal, fasted, and denervated rat skeletal muscle. *Anat. Rec.* 232:60–77.
- Lusk, J. L., J. A. Fox, T. C. Schroeder, J. Mintert, and M. Koohmaraie. 2001. In-store valuation of steak tenderness. *Am. J. Agric. Econ.* 83:539–550.
- Marsh, B. B., and W. A. Carse. 1974. Meat tenderness and the sliding-filament hypothesis. *J. Food Technol.* 9:129–139.
- Marsh, B. B., N. G. Leet, and M. R. Dickson. 1974. The ultrastructure and tenderness of highly cold-shortened muscle. *J. Food Technol.* 9:141–147.
- Miller, M. F., K. L. Huffman, S. Y. Gilbert, L. L. Hamman, and C. B. Ramsey. 1995. Retail consumer acceptance of beef tenderized with calcium chloride. *J. Anim. Sci.* 73:2308–2314.
- Muroya, S., S. Kitamura, S. Tanabe, T. Nishimura, I. Nakajima, and K. Chikuni. 2004. N-terminal amino acid sequences of troponin-T fragments, including 30 kDa one, produced during postmortem aging of bovine longissimus muscle. *Meat Sci.* 67:19–24.
- Muroya, S., I. Nakajima, M. Oe, and K. Chikuni. 2006. Difference in postmortem degradation pattern among troponin-T isoforms expressed in bovine longissimus, diaphragm, and masseter muscles. *Meat Sci.* 72:245–251.
- Negishi, H., E. Yamamoto, and T. Kuwata. 1996. The origin of the 30 kDa component appearing during post-mortem ageing of bovine muscle. *Meat Sci.* 42:289–303.
- Page, S. G., and H. E. Huxley. 1963. Filament lengths in striated muscle. *J. Cell Biol.* 19:369–390.
- Penny, I. F., and E. Dransfield. 1979. Relationship between toughness and troponin-T in conditioned beef. *Meat Sci.* 3:135–141.
- Reuter, B. J., D. M. Wulf, and R. J. Maddock. 2002. Mapping intramuscular tenderness variation in four major muscles of the beef round. *J. Anim. Sci.* 80:2594–2599.
- Ringkob, T. P., D. R. Swartz, and M. L. Greaser. 2004. Light microscopy and image analysis of the thin filament lengths utilizing dual probes on beef, chicken, and rabbit myofibrils. *J. Anim. Sci.* 82:1445–1453.
- Shackelford, S. D., T. L. Wheeler, M. K. Meade, J. O. Reagan, B. L. Byrnes, and M. Koohmaraie. 2001. Consumer impressions of tender select beef. *J. Anim. Sci.* 79:2605–2614.
- Swartz, D. R., M. L. Greaser, and B. B. Marsh. 1993. Structural studies of rigor bovine myofibrils using fluorescence microscopy. I. Procedures for purification and modification of bovine muscle proteins for use in fluorescence microscopy. *Meat Sci.* 33:139–155.
- Trinick, J. 1991. Elastic filaments and giant proteins in muscle. *Curr. Opin. Cell Biol.* 3:112–119.
- Warner, R. D., R. G. Kauffman, and M. L. Greaser. 1997. Muscle protein changes post mortem in relation to pork quality traits. *Meat Sci.* 45:339–352.
- Wheeler, T. L., and M. Koohmaraie. 1994. Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *J. Anim. Sci.* 72:1232–1238.
- Wheeler, T. L., and M. Koohmaraie. 1999. The extent of proteolysis is independent of sarcomere length in lamb longissimus and psoas major. *J. Anim. Sci.* 77:2444–2451.

References

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